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Synthesis of $^2H_3$-Labelled Misoprostol and its Primary Plasma Metabolite

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Summary

Misoprostol-d_3 (**15**) and its major plasma metabolite misoprostol free acid-d_3 (**5**) were synthesized starting from commercially available methyl-d_3-magnesium iodide.

Key words: Prostaglandins, deuterium, misoprostol-d_3, misoprostol free acid-d_3

Introduction

The orally active prostaglandin E_1 analog misoprostol (Cytotec®) is used as an antiulcer drug (1, 2). New indications have recently been reported (3, 4). As part of our ongoing work it was necessary to measure levels of misoprostol (**1**) and its major plasma metabolite, misoprostol acid (**2**), in biological fluids. Misoprostol (**1**) is rapidly absorbed and converted in plasma to its biologically active metabolite **2**. It reaches peak plasma levels in less that 20 minutes (5). Further metabolism

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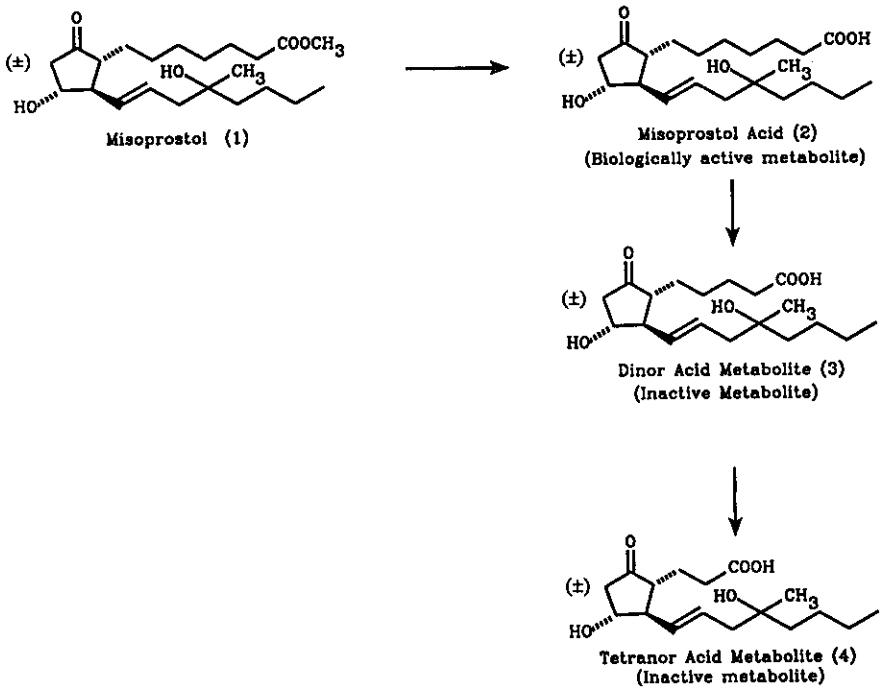
produces inactive compounds 3 and 4 (Scheme 1). Gas chromatography-mass spectrometry (6) or liquid chromatography-electrospray-mass spectrometry are among the most specific and particularly sensitive methods for the measurement of eicosanoids in the picogram ml^{-1} levels. High-performance liquid chromatography combined with atmospheric pressure electrospray ionization mass spectrometry has several advantages: derivatisation of eicosanoids are not necessary, relative complex samples can be analyzed and the pseudomolecular parent ion is the main ion generated. These are important factors for the simplicity, specificity and sensitivity of the analysis (7). A basic requirement for a precise quantitative assay is the availability of a suitable labelled internal standard.

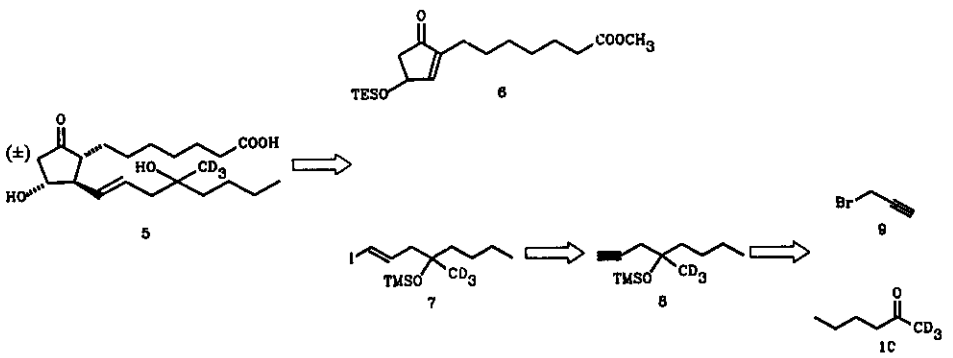
Sheme 1. Metabolism of misoprostol (Cytotec®) in humans.



In this paper we describe the first synthesis of misoprostol-d_3 (15) and its deuterated plasma metabolite 5 via a two component coupling using the dilithiocyanocuprate technology (8-11). As

indicated in Scheme 1, misoprostol is a mixture of two racemates. Although the cuprate reaction is stereospecific, the use of cyclopentenone 6 and side chain 7 , both racemic, produces two racemates. The synthesis is based on the retrosynthetic Scheme 2 where labelling has been considered in a chemically and metabolically stable position. The synthesis starts with the commercially available methyl-d_3-magnesium iodide (11) that is converted in 5 steps to the key intermediate 7 with an extent of trideuteration, estimated by ^{1}H-NMR, greater than 99%. Other routes introducing the label at a later stage resulted in complex mixtures and low yields.

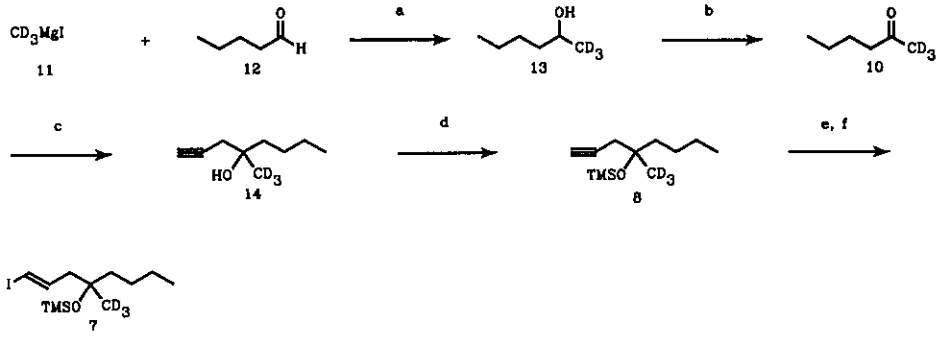
Scheme 2. Retrosynthetic analysis of misoprostol acid-d_3.



Results and Discussion

Grignard reaction of valeraldehyde (12) with methyl-d_3-magnesium iodide (11) afforded the 2-hexanol-1,1,1-d_3 (13) that was oxidized with Jones reagent to 2-hexanone-1,1,1-d_3 (10) (12). A second Grignard reaction with propargylmagnesium bromide, as described by Chen (13), produced 4-(methyl-d_3)-1-octyn-4-ol (14). The hydroxy group in 14 was protected with trimethylsilyl chloride in dimethylformamide, using Corey's procedure (14), to give 8 in high yield. Compound 7 was synthezised from 8 according to Dygos procedure (10). Hydrozirconation of the triple bond of 8, by in situ generated $Cp_2Zr(H)Cl$ (Schwartz' reagent) (15), followed by treatment with iodine gave the (E)-vinyl iodide 7 in good overall yield. (Scheme 3)

Scheme 3. Synthesis of the trideuterated ω-side chain-d_3 7.

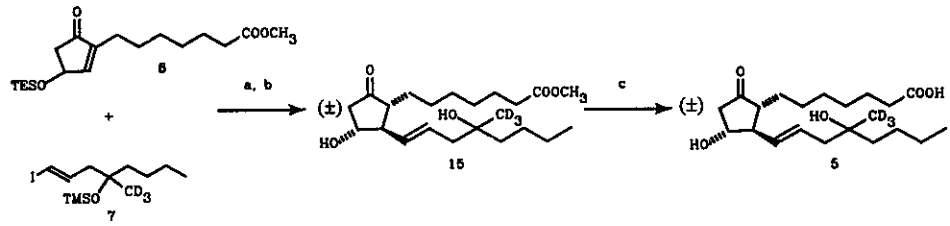


Reagents and conditions: (a) Ether, 0°C–reflux, 2 h; (b) 1.6 M Jones reagent, Acetone, 0°C, 10 min; (c) Mg, $HC{\equiv}CCH_2Br$, cat. $HgCl_2$, Ether/THF; (d) TMSCl, Et_3N, Imidazole, DMF, 0°C; (e) Cp_2ZrCl_2, $LiEt_3BH$, THF; (f) I_2, THF, 0°C.

Two component coupling of compounds 6 (2) and 7 using the dilithiocyanocuprate technology (8-11) gave the 1,4-addition product which after mild desilylation provided misoprostol-d_3 (15). A small amount (3-10%) of the 8-epi-misoprostol-d_3 was coproduced, however separation was easily achieved by chromatography. The final enzymatic ester cleavage using PPL (porcine pancreatic lipase) produced pure misoprostol acid-d_3 (5) in high yield. (Scheme 4)

The misoprostol-d_3 (15) and misoprostol acid-d_3 (5) synthesized were analyzed by HPLC, and ^{1}H- and ^{13}C-NMR. Mass spectrometry analysis (HPLC-API/ES-MS) revealed that 5 and 15 had an extent of trideuteration greater than 99%.

Scheme 4. Synthesis of misoprostol-d_3 (15) and misoprostol acid-d_3 (5).



Reagents and conditions: (a) nBuLi, CuCN, MeLi, Ether -78°C; (b) PPTS, Acetone/H_2O; (c) Lipase (PPL), NaCl, $CaCl_2$, H_2O/THF, pH=7 (kept with 0.1N NaOH).

Misoprostol acid-d_3 (5) was used as internal standard for the quantification of the contents of misoprostol acid in human plasma samples by HPLC-API/ES-MS. Calibration curves were obtained in the range of interest for the pharmacokinetic studies and gave good linear correlation. The detection limit of the method was 10 pg/ml plasma (7).

Experimental

^{1}H-NMR and ^{13}C-NMR (17) data were recorded on a 300 MHz Varian Gemini 2000 Broadband High-Resolution NMR. The progress of the reactions were checked by thin layer chromatography (TLC) using E. Merck silica gel 60F glass plates (0.25 mm). The spots were visualized with UV light, followed by heat staining with p-anisaldehyde in ethanol/sulfuric acid. IR spectra were measured on a Perkin-Elmer Paragon 1000 FT-IR spectrometer. HPLC analysis were performed on a Hewlett-Packard liquid chromatograph HP-1090 Series II with PV5 SDS (Solvent Delivery System) and DAD (Diode Array Detector) equipped with heated column compartment and an automatic liquid injector, or on a Waters HPLC system (M-6000A pump, M-730 Data Module integrator, U6K Injector) and a Schoeffel SF-770 U.V. detector. Silica gel 60 from EM-Science was used for flash chromatography purifications. Mass Spectra were obtained using Hewlett Packard HP-59987A API-Electrospray (Atmospheric Pressure Ionization Electrospray) interface coupled to a Mass Spectrometer Hewlett Packard HP-5989B MS.

4-(Methyl-d_3)-1-octyn-4-ol (14). According to the procedure of Chen (13) **10** was converted to **14** and used in the next step without further purification. *^{1}H-NMR* (300 MHz, $CDCl_3$): δ 2.4-2.3 (2dd AB system, 2H, J=16.5, 2.7 Hz, $C\underline{H}_2$-C≡), 2.1 (t, 1H, J=2.7 Hz, HC≡), 1.6-1.5 (m, 2H, $C\underline{H}_2$-$(CH_2)_2$-CH_3), 1.4-1.2 (m, 4H, $(C\underline{H}_2)_2$-CH_3), 0.9 (t, 3H, J=8.0 Hz, CH_3). *^{13}C-NMR* (75.5 MHz, $CDCl_3$,): δ 80.9 (≡$\underline{C}$-CH_2), 71.0 (HC≡), 67.8 (C), 40.7 ($\underline{C}H_2$-$(CH_2)_2$-CH_3), 32.2 (CH_2-≡), 26.0 (CH_2), 23.0 (CH_2), 13.9 (CH_3). *IR* (NaCl, film): 3422, 3311, 2957, 2935, 2872, 2863, 2228, 2119, 1467, 1380, 1143, 1045, 1025 cm^{-1}.

4-(Methyl-d_3)-4-[(trimethylsilyl)oxy]-1-octyne (8). The silyl ether 8 was obtained according to Corey's method (13, 14) in 67% yield from 10 (two-steps). B.p. 68-75 °C/2 mm. *^{1}H-NMR* (300 MHz, $CDCl_3$): δ 2.4-2.3 (2dd AB system, 2H, J=16.5, 2.7 Hz, CH_2-C≡), 2.0 (t, 1H, J=2.7 Hz, HC≡), 1.6-1.5 (m, 2H, CH_2-$(CH_2)_2$-CH_3), 1.4-1.2 (m, 4H, $(CH_2)_2$-CH_3), 0.9 (t, 3H, CH_3), 0.1 (s, 9H, CH_3-Si). *^{13}C-NMR* (75.5 MHz, $CDCl_3$): δ 82.0 (≡C-CH_2), 75.2 (HC≡), 69.8 (C), 41.4 (CH_2-$(CH_2)_2$-CH_3), 32.6 (CH_2-C≡), 25.9 (CH_2), 23.0 (CH_2), 14.0 (CH_3), 2.3 (3C, CH_3-Si). *IR* (NaCl, film): 3313, 2957, 2937, 2863, 2229, 2120, 1468, 1436, 1251, 1143, 1128, 1051, 980, 839, 753 cm^{-1}.

1-Iodo-4-(methyl-d_3)-4-[(trimethylsilyl)oxy]-1(E)-octene (7). Compound 8 was converted to 7 similar to Dygos (10). Zirconocene chloride hydride was generated in situ following Lipshutz procedure (15) (88% yield). *^{1}H-NMR* (300 MHz, $CDCl_3$): δ 6.5 (dt, 1H, J=14.5, 7.6 Hz, =CH-CH_2), 6.0 (dt, 1H, J=14.5, 1.4 Hz, =CH-I), 2.4-2.2 (2ddd AB system, 2H, J=14.0, 7.6, 1.4 Hz, CH_2-CH=), 1.4 (m, 2H, CH_2-$(CH_2)_2$-CH_3), 1.3-1.2 (m, 4H, $(CH_2)_2$-CH_3), 0.9 (t, 3H, J=6.8 Hz, CH_3), 0.1 (s, 9H, CH_3-Si). *^{13}C-NMR* (75.5 MHz, $CDCl_3$): δ 143.3 (=CH-CH_2), 76.1 (=CH-I), 75.2 (C), 48.7 (CH_2-CH=), 42.2 (CH_2-$(CH_2)_2$-CH_3), 26.1 (CH_2-CH_2-CH_3), 23.1 (CH_2-CH_3), 14.1 (CH_3), 2.6(3C, CH_3-Si). *IR* (NaCl, film): 3050, 2956, 2935, 2862, 2226, 1605, 1466, 1435, 1250, 1077, 1050, 949, 838 cm^{-1}.

Misoprostol-d_3 (15). As described for the synthesis of enisoprost (10) 7 (2.1 g, 6.1 mmol) was coupled with 6 (1.09 g, 3.07 mmol) affording 15 (1.01 g, 85%). *^{1}H-NMR* (300 MHz, $CDCl_3$): δ 5.8-5.7 (m, 1H, =CH-CH_2), 5.4 (2dd, 1H, J=15.0, 8.7 Hz, =CH-CH), 4.0 (apparent q, 1H, CH-OH), 3.7 (s, 3H, CH_3O), 2.7 (ddd, 1H, J=18.3, 7.5, 1.2 Hz, 1H CH_2-CO), 2.5 (br d, 1H, HO-CH), 2.4 (dt, 1H, J=12.0, 8.7 Hz, CH-CH=), 2.3 (t, 2H, J=7.5 Hz, CH_2-COO), 2.3-2.2 (m, 2H, CH_2-CH=), 2.2 (dd, 1H, J=18.3, 9.6 Hz, 1H CH_2-CO), 2.1-1.9 (m, 1H, CH-CO), 1.7-1.4 (m, 6H, CH_2-CH_2-COO, CH_2-CH-CO, CH_2-$(CH_2)_2$), 1.4-1.2 (m, 10H, $(CH_2)_3$-$(CH_2)_2$-COO, $(CH_2)_2$-CH_3), 0.9 (br t, 3H, J=6.9 Hz, CH_3). *^{13}C-NMR* (75.5 MHz, $CDCl_3$): δ 214.9 (CO), 174.4 (COO), 133.5 (=CH-CH), 130.1 and 130.0 (=CH-CH_2), 72.2 (C), 72.1 (CH-OH), 54.9 and 54.8 (CH-CH=), 54.5 (CH-CO), 51.4 (CH_3O), 46.0 (CH_2-CO), 44.9 (CH_2-CH=), 42.0 and 41.4 (CH_2-C), 33.9 (CH_2-COO), 29.3 and 28.8 ($(CH_2)_2$-$(CH_2)_2$-COO), 27.6 (CH_2-CH-CO), 26.5 (CH_2-$(CH_2)_4$-COO), 26.0 and 25.9 (CH_2-CH_2-CH_3), 24.7 (CH_2-CH_2-COO), 23.1 (CH_2-CH_3), 14.0 (CH_3). The frequencies of some of the ^{13}C are different for

every racemate, the underlined values correspond to the racemate that has a smaller retention time in straight-phase HPLC. *IR* (NaCl, film): 3420 (br), 2930, 2857, 1740, 1736, 1459, 1438, 1165, 1077, 973 cm^{-1}. Mass spectral analysis revealed that **15** was >99% trideuterated (area of the ion $[M_{d3\text{-}misoprostol}+Na^+]^+$ at m/e 408 versus area of the ion $[M_{misoprostol}+Na^+]^+$ at m/z 405).

Misoprostol acid-d_3 (5). To a suspension of porcine pancreatic lipase (60 mg) (Type II EC 3.1.1.3), NaCl (5 mg) and $CaCl_2$ (1.5 mg) in water (15 ml) adjusted at pH 7.1, misoprostol-d_3 (**15**; 1 g, 2.69 mmol) in THF (2.5 ml) was added. The pH of the solution was maintained between 7.0-7.2 with 0.1 N NaOH solution. After 30 minutes without pH changes, the solvent was removed under vacuum. The residue was layered with ethyl acetate, and solid potassium bisulfate and sodium chloride were added. The free acid (**5**) was extracted three times with ethyl acetate. Drying over sodium sulfate and concentrating under vacuo afforded misoprostol acid-d_3 (**5**) (0.9 g, 90%). *^{1}H-NMR* (300 MHz, $CDCl_3$): δ 5.7 (m, 1H, =C<u>H</u>-CH_2), 5.4 (dd, 1H, J=15.0, 8.5 Hz, =C<u>H</u>-CH), 4.0 (apparent q, 1H, C<u>H</u>-OH), 2.7 (dd, 1H, J=18.6, 7.5 Hz, 1H CH_2-CO), 2.4-2.1 (m, 4H, C<u>H</u>-CH=, C<u>H</u>$_2$-CH=, 1H CH_2-CO), 2.3 (t, 2H, J=7.3 Hz, CH_2-COO), 2.0 (m, 1H, CH-CO), 1.7-1.2 (m, 16H, (C<u>H</u>$_2$)$_5$-CH_2-COO, (C<u>H</u>$_2$)$_2$-CH_3, CH_2-C), 0.9 (t, 3H, J=6.6 Hz, CH_3). *^{13}C-NMR* (75.5 MHz, $CDCl_3$): δ 215.6 (CO), 178.7 (COO), 133.8 (=<u>C</u>H-CH), 129.6 and 129.5 (=<u>C</u>H-CH_2), 72.8 and 72.7 (C), 71.8 (CH-OH), 54.7 and 54.6 (<u>C</u>H-CH=), 54.5 (<u>C</u>H-CO), 46.1 (<u>C</u>H_2-CO), 44.6 (<u>C</u>H_2-CH=), 42.0 and 41.0 (<u>C</u>H_2-C), 33.8 (<u>C</u>H_2-COO), 29.1 and 28.5 ((<u>C</u>H_2)$_2$-$(CH_2)_2$-COO), 27.3 (<u>C</u>H_2-CH-CO), 26.2 (<u>C</u>H_2-$(CH_2)_4$-COO), 26.1 and 25.9 (<u>C</u>H_2-CH_2-CH_3), 24.4 (<u>C</u>H_2-CH_2-COO), 23.1 (<u>C</u>H_2-CH_3), 13.9 (CH_3). *IR* (NaCl, film): 3401 (br), 2929, 2857, 1738, 1714, 1463, 1263, 1161, 1075, 1045, 972 cm^{-1}. Mass spectral analysis revealed that **5** was >99% trideuterated (area of the ion $[M_{d3\text{-}misoprostol\ acid}\text{-}H^+]^-$ at m/e 370 versus area of the ion $[M_{misoprostol\ acid}\text{-}H^+]^-$ at m/z 367).

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16. The ^{13}C signal (septuplet) corresponding to the carbon CD_3 was sometimes not observed due to its low intensity.

